Physical and Numerical Design of a Fluidised Bed Bioreactor for Stem Cell Expansion

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Introduction

Bone substitutes with enhanced biological activity are required for the replacement, repair and regeneration of skeletal tissue. This project aims to design a bioreactor for stem cell expansion on novel hydroxyapatite (HA/TCP) bone substitute materials designed for accelerated osseointegration of implants. The substitute material will be porous to allow cell expansion throughout. Mathematical modelling has been used alongside physical and biological experiments to define the bioreactor environment.

Bone Substitute Material

Porous bone substitute material has been produced from hydroxyapatite and tricalcium phosphate (HA/TCP)[1]. Figure 1 shows a photograph of a porous particle made from HA/TCP. The aim of this project is to seed this material with bone cells and then grow them in a fluidised bed bioreactor, a method which allows both good mixing and transport of nutrients and also causes some shear on the cells. Figures 2 and 3 show SEM images of a porous particle. The surface comprises individual crystals of HA/TCP which can also be seen in Figure 4, an SEM image of a flat disk of the same HA/TCP material, produced in a press, that will be used in static cell expansion experiments. Figure 5 shows an SEM image of some MG63 cells grown on a flat disk of HA/TCP. The cells in the image appear to have left a thin coating on the ceramic surface, which is partially obscuring the crystal structure seen in Figures 3 and 4.

Figure 1: A porous HA/TCP particle. Particle dimensions are approximately 5x5x5 mm

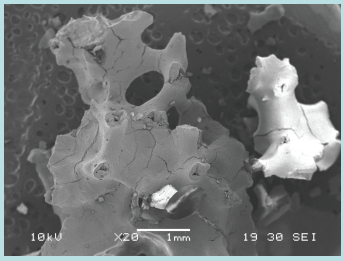

Figure 2: An SEM image of a porous HA/TCP particle (20x magnification)

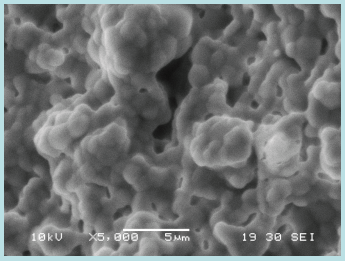

Figure 3: An SEM image of a porous HA/TCP particle (5000x magnification)

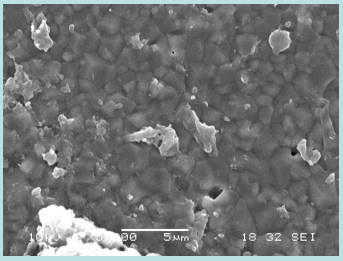

Figure 4: An SEM image of a flat disk of HA/TCP (5000x magnification)

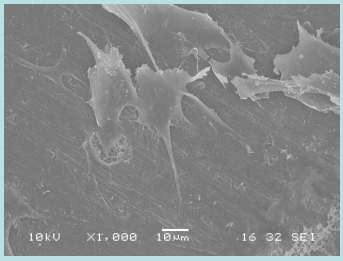

Figure 5: An SEM image of MG63 cells growing on a flat disk of HA/TCP (1000x magnification)

Fluidisation of Bone Substitute Material

Initial tests have been performed to fluidise the porous bone substitute material shown in Figures 1, 2 and 3. Figures 6 – 8 show images of the particles being fluidised in a 50 mm diameter column. The distributor comprised 1 mm holes in a non-optimised pattern. In Figure 6, the flow rate is insufficient to fluidise the particles. In Figure 7 the flow rate has been increased and the bed has expanded, the gaps between the particles can be seen to have increased in size. In Figure 8 the flow rate has been increased again and has resulted in uneven expansion, with the left hand side of the column higher than the right.

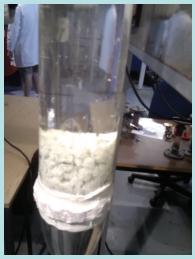

Figure 6: A bed of porous particles at a fluid velocity below that of initial fluidisation

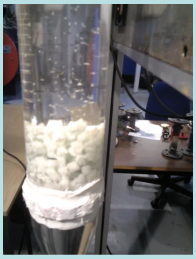

Figure 7: A bed of porous particles at a fluid velocity causing initial fluidisation

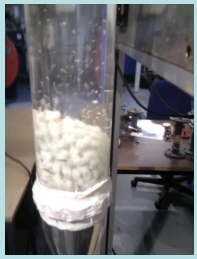

Figure 8: A bed of porous particles at a fluid velocity causing asymmetric fluidisation

Fluidisation of these porous particles was hampered by their shape. A spherical particle will readily brush past another particle, whereas the shape of these particles encourages interlocking. This resulted in the bed appearing to stop moving at times during the fluidisation, before an increase in the upward velocity of the liquid caused the particles to unlock and re-fluidise.

The velocities required to achieve fluidisation were higher than those predicted by the modelling (see right). This is due to a combination of factors: the model is for spherical particles, the interlocking, a non-optimised distributor.

Modelling of Distributor and Fluidisation

A CFD model of the distributor and fluidisation is being developed to enable aspects of the design to be optimised outside of the laboratory. Figures 9 – 11 show a model of a distributor in a 25 mm diameter column. The 3 mm thick distributor is placed 10 mm above the narrow inlet nozzle to improve the distribution of the liquid across the column.

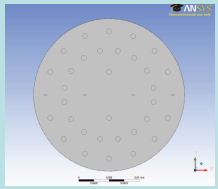

Figure 9: Top view of distributor depicting layout of holes

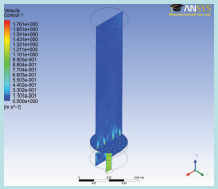

Figure 10: Isometric view of column with velocity profiles

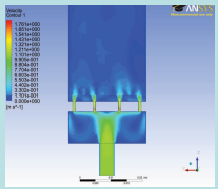

Figure 11: Side view of column with velocity profile

Figures 12 – 14 show a cross-section of a model of a 50 mm diameter column containing two phases. The solid phase volume fraction is shown in the figures. The solid phase was modelled as an Eulerian continuum of 5 mm spherical particles. The superficial liquid velocities passing up through the column are 0.03, 0.08 and 0.12 ms^{-1} respectively. It can be seen that as the velocity is increased the particles begin to fluidise within the column. The next stage of modelling will be to combine the design of the distributor with the fluidisation, so that the inlet of the fluidisation column is represented by the flow profile at the top of the distributor.

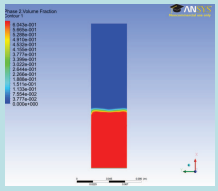

Figure 12: Volume fraction of solid phase, velocity of liquid phase = 0.03 ms^{-1}

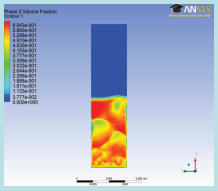

Figure 13: Volume fraction of solid phase, velocity of liquid phase = 0.08 ms^{-1}

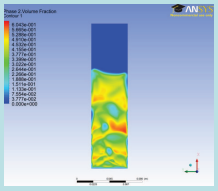

Figure 14: Volume fraction of solid phase, velocity of liquid phase = 0.12 ms^{-1}

Conclusions

- The SEM images show that cell expansion on this material is possible. Cell expansion on the porous particles, as opposed to the flat disks, will also be dependent on the cells' interaction with the surface which shows greater undulation, but a similar structure to the flat disks.
- Fluidisation of the particles was achieved, but suffered problems due to the interlocking nature of the particles. This could be overcome with a larger diameter column, an optimised distributor or by applying mechanical force to the particles to cause any large protuberances to be removed, thus reducing interlocking.
- The modelling has shown that the velocity required to fluidise spherical particles of similar size and density to the porous particles underestimated the velocity required. The next stage of the modelling process will be to adapt the model to account for this difference.

References

1. Hsu, Y.H. *et al.* J. Materials Sci.: Mat. in Med. **18**, 1931-1937 (2007)